

WHAT IS CLAIMED IS:

1. A process for detecting a single nucleotide polymorphism (SNP) comprising:

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(a) contacting one or more allele specific oligonucleotide primers (P1) with one or more target polynucleotides (TP), wherein said target polynucleotide possesses a first portion that is complementary to a second portion located on said P1 at or near one end thereof but wherein the terminal nucleotide, and third nucleotide from the terminal nucleotide, at said end of said P1 may not be complementary to the corresponding nucleotide of said target polynucleotide, and wherein such contacting occurs under conditions that promote hybridization between the first and second portions thereby forming an P1-TP complex;

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(b) contacting the P1-TP complex of (a) with an exonuclease deficient deoxyribonucleotide (DNA) polymerase enzyme under conditions that promote extension of the P1 with the TP as template thereby forming an extended segment (ES) of P1; and

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(c) detecting the extended P1.

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2. A process for detecting the extended P1 of claim 1 comprising the further steps of:

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(d) removing the target polynucleotide (TP) from the complex formed in step (b);

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(e) contacting a primer oligonucleotide (P2) with the extended P1, wherein the primer oligonucleotide comprises a first segment complementary to at least a portion of the extended segment (ES) formed in step (b) and a second segment

that includes the 3'-terminus of said primer oligonucleotide (P2) under conditions promoting hybridization of P2 and the extended P1 (EP1) to form an EP1-P2 complex;

5 (f) contacting an amplification target circle (ATC) with the EP1-P2 complex under conditions that promote hybridization between the amplification target circle and the P2 portion of said EP1-P2 complex to form an EP1-P2-ATC complex; and

10 (g) contacting DNA polymerase with the EP1-P2-ATC complex under conditions that promote replication of the amplification target circle,

wherein said replication of the ATC results in the formation of tandem sequence DNA (TS-DNA) thereby indicating the extension of P1.

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3. The process of claim 2 wherein the target polynucleotide is derived from genomic DNA.

4. The process of claim 2 wherein the DNA is genomic DNA.

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5. The process of claim 4 wherein the genomic DNA is human genomic DNA.

25 6. The process of claim 4 wherein the genomic DNA is non-human genomic DNA.

7. The process of claim 2 wherein the target DNA is a mixture of human and non-human genomic DNA.

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8. The process of claim 2 wherein the DNA polymerase of step (g) is an enzyme selected from the group consisting of bacteriophage  $\phi$ 29 DNA

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A2  
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A3

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polymerase, phage M2 DNA polymerase, phage  $\phi$ -PRD1 DNA polymerase, VENT<sup>®</sup> DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase, *E. coli* DNA polymerase III holoenzyme, Tts polymerase and T7 DNA polymerase.

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9. The process of claim 1 wherein the exonuclease-deficient DNA polymerase is T7 Sequenase or Tth polymerase.

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10 exonuclease deficient.

11. The process of claim 10 wherein the DNA polymerase is an enzyme selected from the group consisting of Klenow polymerase (exo<sup>-</sup>), Vent polymerase (exo<sup>-</sup>), Deep Vent polymerase (exo<sup>-</sup>), Pfu polymerase (exo<sup>-</sup>), Taq polymerase, the Stoffel fragment of Taq polymerase, Bst polymerase, Tts polymerase, and ThermoSequenase.

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12. The process of claim 1 wherein at least one end of the allele specific oligonucleotide primers (P1) is attached to a solid support.

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13. The process of claim 12 wherein the solid support is composed of at least one member selected from the group consisting of acrylamide, cellulose, nitrocellulose, glass, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids.

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14. The process of claim 12 wherein said solid support is made of glass or plastic.

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15. The process of claim 1 wherein the allele specific oligonucleotide primer (P1) is selected from the group consisting of the sequences of SEQ ID NOs: 1, 2, 3, 4, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26.

5        16. The process of claim 2 wherein said primer (P2) comprises an oligonucleotide having a sequence selected from the group consisting of SEQ ID NO: 27, 28, 34 and 35 wherein each said sequence has been converted to bipolar form.

10        17. The process of claim 2 wherein said ATC comprises a polynucleotide having a sequence selected from the group consisting of SEQ ID NO: 29 and 30.

15        18. A method for diagnosing a disease characterized by a genetic mutation comprising:

(a) obtaining a sample of a mutated gene sequence from an organism afflicted with said disease; and

20        (b) carrying out the process of claim 1 wherein at least a portion of said mutated gene sequence is used as either the target polynucleotide or the allele specific oligonucleotide.

25        19. The process of claim 18 wherein the mutated gene sequence is used as the target polynucleotide.

20. The process of claim 18 wherein said animal is a human.

30        21. The process of claim 18 wherein said disease is a disease caused by, induced by or related to a mutation in at least one gene.

22. The process of claim 21 wherein said disease is a member selected from the group consisting of Parkinson's disease, Duchenne muscular dystrophy, Niemann-Pick disease, polyposis, neurofibromatosis, polycystic kidney disease, Tay-Sachs disease, xeroderma pigmentosa, ataxia-telangiectasia, Huntington disease, Li-Fraumeni syndrome, beta-thalassemia, sickle cell anemia, hemoglobin C disease, hemophilia, acute intermittent porphyria, cystic fibrosis, diabetes, obesity and cancer.

23. The process of claim 22 wherein said cancer is a member selected from the group consisting of leukemia, lymphoma, melanoma, neuroblastoma, retinoblastoma, rhabdomyosarcoma, Ewing sarcoma, head and neck cancer, skin cancer, brain cancer, esophageal cancer, stomach cancer, lung cancer, breast cancer, colon cancer, ovarian cancer, testicular cancer and prostate cancer.

24. The process of claim 1 wherein the third nucleotide from the end of said P1 is complementary to the corresponding nucleotide of the target polynucleotide.

25. The process of claim 2 wherein the third nucleotide from the end of said P1 is complementary to the corresponding nucleotide of the target polynucleotide.

26. The process of claim 1 wherein the third nucleotide from the end of said P1 is not complementary to the corresponding nucleotide of the target polynucleotide.

27. The process of claim 2 wherein the third nucleotide from the end of said P1 is not complementary to the corresponding nucleotide of the target polynucleotide.

28. The process of claim 1 wherein each of the terminal nucleotide and third nucleotide from the end of said P1 is not complementary to the corresponding nucleotide of the target polynucleotide.

5 29. The process of claim 2 wherein each of the terminal nucleotide and third nucleotide from the end of said P1 is not complementary to the corresponding nucleotide of the target polynucleotide.

30. A kit, comprising:

10 (a) a plurality of oligonucleotide probes, each oligonucleotide probe of the plurality being capable of hybridizing to one or more target polynucleotides that may or may not possess a mismatch with respect to a terminal residue of the oligonucleotide probes;

(b) a sample of an exonuclease deficient DNA polymerase;

15 (c) a plurality of amplification primers, each said primer being capable of hybridizing to an elongated segment of said oligonucleotide probe as well as comprising a primer sequence complementary to a sequence on an amplification target circle (ATC) for use in rolling circle amplification;

(d) a sample of one or more amplification target circles (ATC), essentially  
20 single stranded DNA circles, each comprising a sequence of 10 to 20, even 30, nucleotides in length, which sequence is complementary to a sequence of the amplification primers of part (c) and which ATCs act as templates for rolling circle amplification (RCA);

(e) a sample of a DNA polymerase capable of carrying out rounds of  
25 rolling circle amplification;

(f) a means for detecting the products of rolling circle amplification, including, but not limited to, various labeling reagents and address probes; and

(g) a set of instructions for carrying out the steps of claim 1 and claim 2.

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